

## **Mixtures of 17 $\beta$ -Trenbolone with Ethinylestradiol or Bisphenol A Altered Tubercle Formation and Steroid Production in the Fathead Minnow**

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The xenoestrogens, ethinylestradiol (EE2) and bisphenol A (BPA), and the androgen 17 $\beta$ -trenbolone (TRB) are examples of endocrine disrupting compounds (EDCs) commonly detected in the environment. All have been shown to affect fish reproductive endocrinology individually, but little is known about possible effects of mixtures of the chemicals. Two studies were conducted to examine the effects of EE2 or BPA and TRB mixtures on tubercle development, vitellogenin (vtg) production, and steroid production in *Pimephales promelas* (fathead minnow). These experiments used a 14-d flow-through water exposure. We measured plasma vtg by enzyme linked immunosorbent assay, ex vivo and plasma steroids by radioimmunoassay, and tubercles were evaluated with a dissecting microscope. EE2 significantly induced vtg in males, in the absence or presence of TRB. In females, TRB alone depressed vtg, and co-treatment with EE2 did not seem to offset this effect. A similar response pattern was observed in males and females from the BPA-TRB experiment. Consistent with its expected androgenic effect, TRB exposure significantly increased tubercle scores of female fish compared to the control; however, the BPA- and EE2-TRB mixtures appeared to offset this response to some degree. In conclusion, environmental EDCs are not found separately but are present as mixtures. Our results indicate that these mixtures can cause unique *in vivo* effects compared to responses seen with individual chemicals.

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